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APPLICATION NUMBER: *60/574,131*
FILING DATE: *May 25, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US04/42948*

Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
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Substitute for PTO/SB/16 (08-03)

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 316084488US

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☐ Additional inventors are being named on the ____ separately numbered sheets attached hereto.

TITLE OF INVENTION (500 characters max)

POLYAMIDES FOR NUCLEIC ACID DELIVERY

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 240 ☐ CD(s), Number

☐ Drawing(s) *Number of Sheets* ☐ Other (specify)

☐ Application Data Sheet. See 37 CFR 1.76

METHOD FOR PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27	**FILING FEE Amount ($)**
☐ A check is enclosed to cover the filing fees	$80.00

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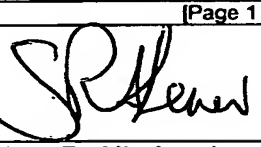
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government

☒No

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE 

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Date May 25, 2004

REGISTRATION NO. 41,487

Docket Number **91830/0521069**

CinLibrary/1401840.1

U.S. PROVISIONAL PATENT APPLICATION

POLYAMIDES FOR NUCLEIC ACID DELIVERY

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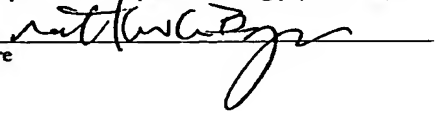
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POLYAMIDES FOR NUCLEIC ACID DELIVERY

FIELD OF THE INVENTION

[0001] Polymeric materials are currently being investigated for the delivery of therapeutic DNA. The use of synthetic delivery agents has many advantages over viral delivery vectors for several reasons such as they may not induce immune and inflammatory responses and thus can be used repeatedly in clinical administration. In addition, synthetic vectors have a lower cost, are easier to manufacture on a larger scale, and they have the ability to carry an unlimited amount of genetic information. Several studies have shown that polycations (polymers that exhibit a positive charge at physiological pH) can bind DNA and successfully transfect many cell types with varying degrees of gene expression (delivery efficiency) and toxicity. Polycations self assemble with DNA through electrostatic interactions and compact DNA into small complexes that have been termed polyplexes. The formation of polyplexes usually occurs at a N/P ratio [the ratio of polymer nitrogens (N) / phosphate groups (P) on DNA] greater than one. Polyplexes can be taken up by cells through the endocytotic pathway. After uptake, some of the polyplexes are able to escape the endosomes and are transported into the nucleus (most likely during cell division) where the delivered gene is transcribed. The polyamides are created from comonomers (x = 1, L-tartrate and x = 2, galactarate). Several studies have indicated that the polymer structure plays a role in both the delivery efficiency and toxicity that is observed during gene transport. Here, we have created a series of polymers that will allow us to probe the structure-property relationships for synthetic vectors. We have created a series of polyamides that vary in the amount of the hydroxyl and secondary amine groups along the polymeric backbone.

BACKGROUND OF THE INVENTION

[0002] Nucleic acids show great promise as new therapeutics to treat both acquired and inherited diseases. One of the greatest challenges with the successful application of nucleic acid drugs is the development of an efficacious delivery method.1 Delivery systems are needed to compact genetic material into nanostructures that can be uptaken by cells, protect nucleic acids from enzymatic damage during cellular transport, and provide the possibility of targeting the delivery to specific cell types.2 Viral vectors are still the most effective and commonly used method of DNA transport even though many problems with this delivery method have been revealed.2,3

[0003] Polymer-mediated gene delivery has recently emerged as a viable alternative to viral-based transfection systems since polymers may not induce immune and inflammatory responses, have a lower cost of synthesis, and have a large nucleic acid loading capacity.1,2 Several studies have shown that polycations bind DNA electrostatically and form polyplexes (polymer + DNA complexes) that are endocytosed by many cell types and deliver DNA with varying degrees of delivery efficiency and toxicity.4,5 Although synthetic delivery systems show great promise, difficulties with polymer toxicity and low delivery efficiency have hampered clinical application of these vectors.1,2 For example, polyethylenimine (PEI), a polymer of ethylenediamine, exhibits efficient gene delivery but is also very cytotoxic.6 Conversely, chitosan, a polymer of glucosamine, is completely nontoxic yet reveals low delivery efficiency in many cell lines.7 Progress towards rationally-designed synthetic delivery systems has also been stalled by a lack of understanding of the fundamental polymer structure-biological property relationships that exist for synthetic delivery vehicles.4,5

SUMMARY OF THE INVENTION

[0004] We have developed a new series of polyamides that show promise as gene delivery agents. The polymers created in this study bind DNA and should facilitate cellular uptake. Particle sizing experiments with the polyplexes and in vitro delivery of plasmid DNA is currently being studied with several mammalian cell lines.

[0005] In addition, we are using transcription factor decoys (including, but not limited to NF-kB), to block signaling and gene expression associated with pathogenesis. The data that we have pertains to blocking NF-kB in the heart, which we have shown is efficacious in reducing myocardial infarction. However, the concept that we wish to disclose takes this several steps further.

[0006] First, we are and will use linear duplications or chains of these decoys, such that each strand contains a number of decoy transcription factor binding sites more than 30 (no upper limit at present). We believe this will stabilize the decoy AND increases efficacy. Secondly, we can incorporate decoys for multiple transcription factors into one of these strands, such that we can affect blockade of 2, 3, or more transcription factors simultaneously in a cell. Third, we use these strands, or the strands contained in a plasmid or other DNA vector (can include phage, viral or other DNA) to bind to the polymers to deliver the strands to the cytoplasm of the cell, to effect transcription factor blockade. The decoys may be any transcription factors, including, but not limited to, NF-kB, AP-1, ATF2, ATF3, SP1 and others. This is all based on the novel concept, supported by data in our lab, that blocking key signaling molecules simultaneously can have additive or even synergistic therapeutic effects, particularly when the molecules chosen are key signaling hubs. In signaling, transcription factors participate by activating or turning down gene expression. Thus, we anticipate and have shown that the effect of transcription factor blockade results in reducing or preventing gene expression changes

associated with pathogenesis. For NF-kB, our proof of principle, two of the genes that we have shown are involved in MI and reduced by blocking NF-kB are iNOS and Cox2. We have preliminary data that others are metallothionein and heat shock protein 70.

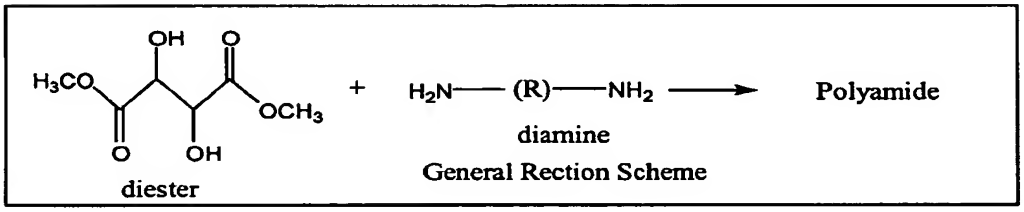
DETAILED DESCRIPTION OF THE INVENTION

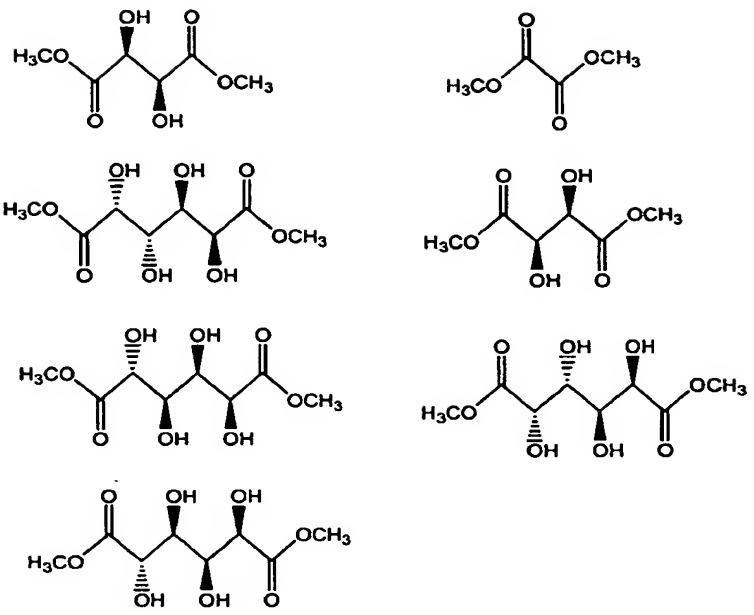
[0007] We have developed a new series of polyamides that show promise as gene delivery agents. The polymers created in this study bind DNA and should facilitate cellular uptake. Particle sizing experiments with the polyplexes and in vitro delivery of plasmid DNA is currently being studied with several mammalian cell lines.

The Polymers

[0008] Polyamides may be prepared by condensation of an appropriately substituted diester and an appropriately substituted diamine comonomer.

[0009] Diesters include, but are not limited to, those shown below, their stereoisomers, mixtures of isomers, and also D-Mannaro-1,4-:6,3-dilactone, dimethyl D-glucarate (linear and closed ring forms of all stereoisomers), methyl citric acid, methyltartronic acid, methyl D-arabinaric acid, esters of xylaric acid and methyl heptaric acid.





Suitable diesters

Suitable diamines include but are not limited to those given in the formula below, where R may be an alklychain incorporating an variety of functional groups including ketones, amines, esters, alcohols, ethers, thiols, thioesters, phosphates, phosphonates. The R group is preferably are alkyl polyamine chain of varying lengths, with examples given in Table 1.

Table 1:

Diamine **NH_2-R-NH_2; where R may be**

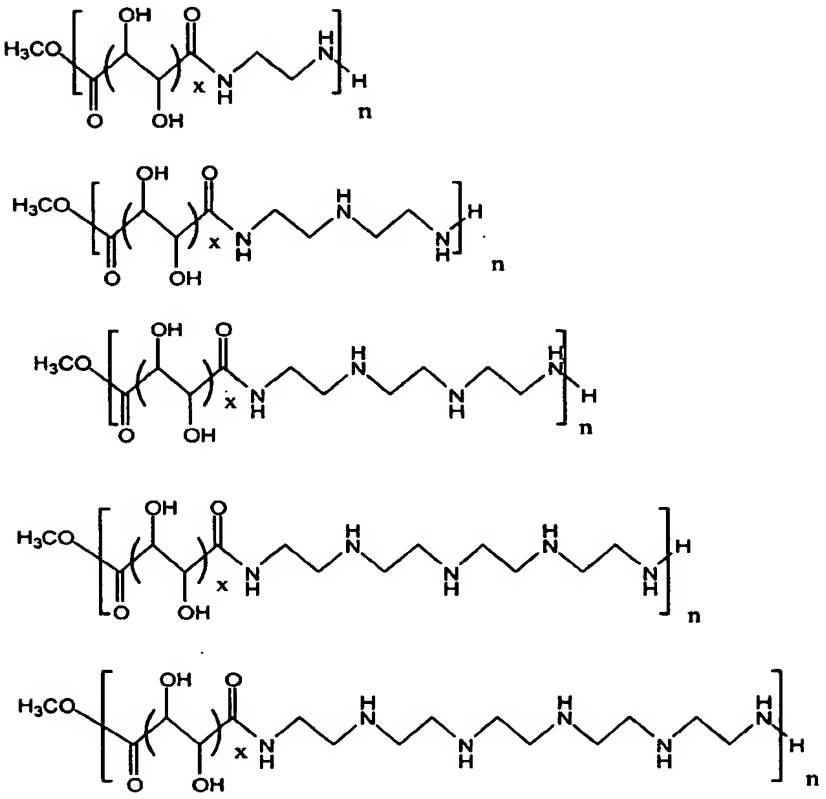
–$(CH_2)_2$- NH-$(CH_2)_2$ - ;

–$(CH_2)_2$ -NH-$(CH_2)_2$- NH-$(CH_2)_2$- ;

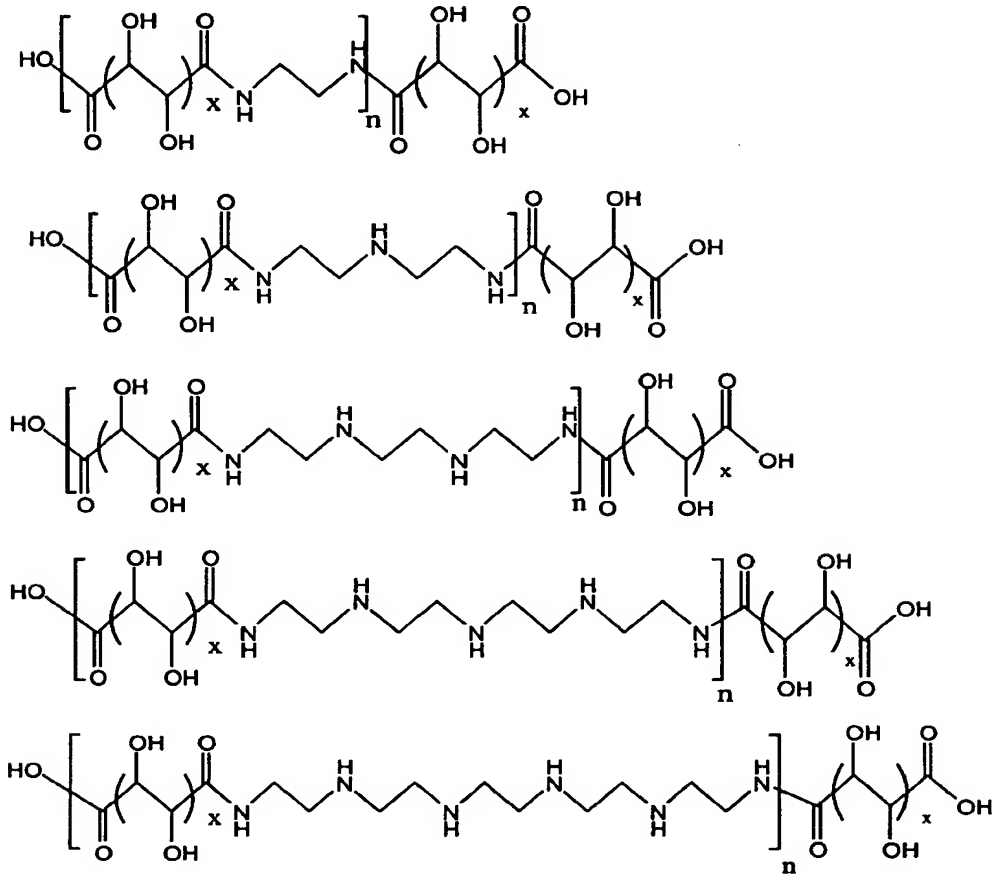
$(CH_2)_2$ -NH-$(CH_2)_2$- NH-$(CH_2)_2$- NH- $(CH_2)_2$-;

$(CH_2)_2$ -NH-$(CH_2)_2$- NH-$(CH_2)_2$- NH- $(CH_2)_2$-NH $(CH_2)_2$;

An example of one class of polyamides of the present invention includes those shown below (wherein x = 1, L-tartrate; and when x = 2, galactarate, and n= 1-50)

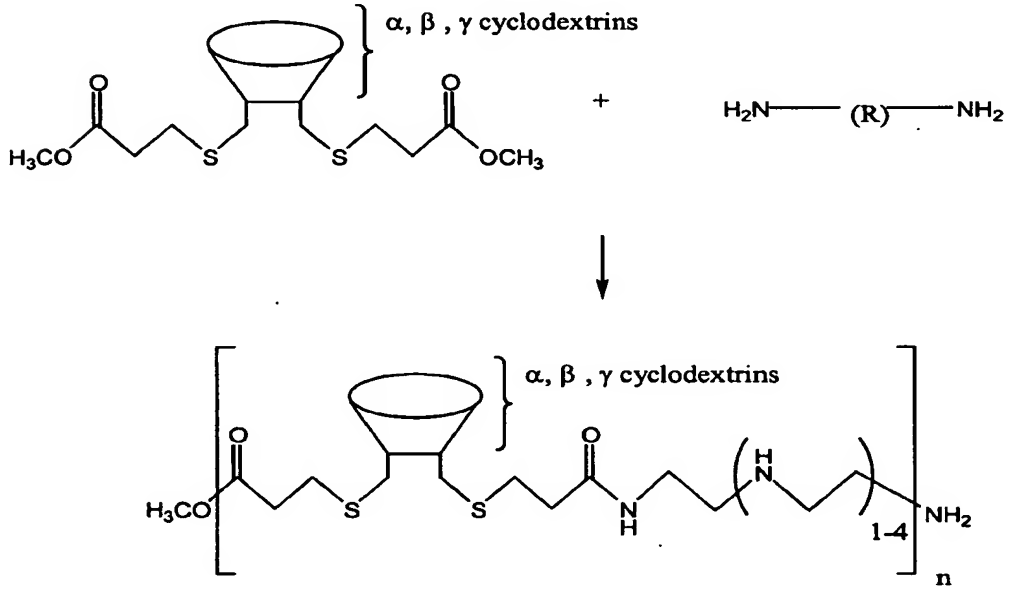


Other classes of polyamides include anhydride-terminated and carboxylic acid-terminated. Examples of a carboxylic acid-terminated polyamides of the present invention includes the following:



wherein x = 1, L-tartrate; and when x = 2, galactarate, and n= 1-50;

Another class of polyamides are those obtained by reacting a cyclodextrin of the formula below with a polyamine, such those give in Table 1, to arrive at a cyclodextrin-polyamide. Suitable cyclodextrins include alpha, beta and gamma cyclodextrins. These cyclodextrins may be substituted in such a way as to produce branched polyamides by reacting with the appropriate polyamine. In such systems, a dedrimer-type array might be formed, in which the amine chains would be attached to the cyclodextrin base.



In addition, most any carbohydrate (sugar) molecule may be utilized as the starting material for this invention, including monosaccharides and disaccharides and polysaccharides. Examples include, but are not limited to, tartrate, galactose, glucose, mannarate, glucarate, trehalose, and mannose. These sugars may be in either tautomeric form, i.e., either the linear molecule or the closed-ring (cyclized) form.

Oligonucleotide Decoys.

[0010] In one embodiment, the present invention relates to the use of oligonucleotide decoys for the production of a medicament for the therapy of NF-κB-dependent diseases. The present invention also relates to the prevention and treatment of various diseases associated with NF-κB which is known to be a regulatory factor in the transcription of cytokines and adhesion factors. More particularly, the invention relates to an NF-κB decoy, a composition comprising the decoy for the therapy and prophylaxis of NF-κB-associated diseases, and a method for the therapy and prophylaxis.

[0011] An important step in many inflammatory processes is the translocation of the protein "nuclear factor kappa B", in brief NF-κB, into the cell nucleus and the stimulation of the expression of the genes caused thereby, whose products are responsible for inflammatory reactions. For example, in asthma the nonbeneficial, excessive (non self-limiting) production of these proteins is responsible for the intensification and maintenance of the inflammatory process and the unpleasant to life-threatening symptoms of this disease associated therewith. Because the long-term treatment with glucocorticoids corresponding to the present state of the art is affected by some disadvantages, NF-κB is seen as a compelling target for the development of new anti-inflammatory active compounds against asthma.

[0012] The oligonucleotide decoy substances utilizable according to the invention are inhibitors which selectively inhibit nuclear factor kappa B (NF-κB)-mediated pathophysiological processes. NF-κB-mediated processes occur in inflammatory diseases, immunological disorders, septic shock, transplant rejection, radiation damage, reperfusion injuries after ischemia, thromboses or in complex, chronic inflammatory disorders such as arteriosclerosis.

[0013] Nuclear factor kappa B (NF-κB) is a dimeric protein complex occurring in many tissue cells and in particular in blood cells. NF-κB takes on a particular role in the control of the expression of genes which have an NF-κB binding sequence (5'-GGGPuNNPyPyCC-3') in their promoter sequence. To this extent, NF-κB is a transcription factor. The physiological activity of NF-κB in the control of gene expression, however, is subject to a regulation principle, in which NF-κB is released from a complex with the protein IκB in order to be translocated as a transcription factor in the cell nucleus of gene activation. The regulation principle for the release of active NF-κB from a complex with the protein IκB is still not known in detail.

[0014] Likewise, it is not known how the formation of homodimeric and heterodimeric NF-κB protein complexes takes place. NF-κB acts on gene activation as a dimeric transcription factor. The dimerization can take place under the structurally related transcription factors Rel A, Rel B, c-Rel, p50 or p52, which form a family of transcription factor proteins. In the dimerization of the subunits to the NF-κB, there can also already be a regulation principle for the control of the genes later described in greater detail, which is still not known.

[0015] A crucial feature of NF-κB compared to other transcription factors is that NF-κB is a primary transcription factor. Primary transcription factors are already present in the cell in inactive (usually complex-bound) form and are released after an appropriate stimulus in order to be able to display their action very rapidly. Primary transcription factors are not first formed by the activation of the associated gene and subsequent transcription and translation.

[0016] This property of NF-κB, the formation of homodimeric or heterodimeric Rel proteins and the formation of an inactive protein complex with an IκB protein, offer very different points of attack for pharmacologically active substances than the points of attack of the de novo biosynthesis of

transcription factors. For the sake of completeness, it may be mentioned that the genes for the formation of NF-κB (genes of the Rel family) and the genes for the formation of the IκB proteins (gene family comprising the genes for IκB-α, IκB-beta, p105/IκB-gamma, p100/IκB-delta, IκB-epsilon and others) for their part are of course also subject to regulation, which can be points of attack for pharmaceutically active substances. Thus it is known that the expression of the constitutively formed IκB proteins p105 and p100 is increased by stimuli which also activate NF-κB, such as tumour necrosis factor-α (TNF-α) or phorbol myristate acetate (PMA).

[0017] A regulation mechanism is described in the literature in which it is shown that the overexpression of IκB binds active NF-κB and thus inactivates it. This also applies if the NF-κB has already entered into a complex with the DNA (P. A. Baeuerle, T. Henkel, Annu. Rev. Immunol. 12, 141-179, 1994). From this it can be concluded that there are a number of specific points of attack in the biochemical function of NF-κB and IκB proteins which should make it possible to inhibit an undesirable, pathophysiological, NF-κB-dependent gene activation selectively.

[0018] A chemical compound which selectively inhibits the function of NF-κB or the function of IκB proteins or IκB genes to an increased extent should be able to be used as a pharmaceutical for the suppression of NF-κB-mediated disease processes.

[0019] Primarily, NF-κB can promote all pathophysiological processes in which genes are involved which have the NF-κB binding sequence in their promoter. Mainly, these are genes which play a crucial causal role in immunological complications, in inflammatory diseases, autoimnmune disorders, septic shock, transplant rejection, thromboses or else alternatively in chronic inflammatory diseases such as arteriosclerosis, arthritis, rheumatism and psoriasis.

[0020] NF-κB binding sequences contain, for example, the promoters of receptors of lymphoid cells (T-cell receptors), of MHCI and MHCII genes, of cell adhesion molecules (ELAM-1, VCAM-1, ICAM-1), of cytokines and growth factors (see also the following table). Furthermore, NF-κB binding sequences are found in the promoters of acute phase proteins (angiotensinogen, complement factors and others).

[0021] A chronically increased or acutely overshooting activation of the genes mentioned leads to various pathophysiological processes and syndromes.

[0022] The rapid and overshooting production of cytokines of the inflammatory reaction (TNFα, interleukin-2, interleukin-6, interleukin-8 and others) and of the adhesion molecules (ELAM-1, ICAM-1, VCAM-1) in leukocytes, in particular in macrophages and also in endothelial cells, is a causal feature of processes which often run a fatal course in the case of septic shock; or in the case of radiation damage and in the case of transplant rejection often leads to considerable complications. Inhibitors which prevent the NF-κB-mediated gene expression intervene very early in some diseases in the expression of pathophysiological changes and can therefore be a very effective therapeutic principle. An example is also NF-κB inhibitors for diseases which are to be attributed to an overexpression of acute-phase proteins. An undesirable overexpression of acute-phase proteins can cause a complex general reaction in which tissue damage of very different types, fever and local symptoms such as inflammation and necroses can occur. Usually, the blood picture is changed. NF-κB strongly induces, for example, the serum amyloid A precursor protein in the liver in the course of induction of acute-phase proteins.

[0023] For example, the NF-κB-mediated gene expression of the interleukin-2-(II-2) gene can be inhibited.

[0024] Interleukin-2 is a cytokine, which plays a central role in various inflammatory processes, inter alia, as a hematopoietic growth factor

(Annu. Rev. Immunol. 1994, 12: 141-79). The promoter of the interleukin-2 gene is NF-κB dependent. An inhibitor of NF-κB stimulation thus opens up the possibility of preventing overshooting of II-2 production and thus of treating inflammatory processes.

[0025] In the case of other syndromes such as tissue damage after reperfusion or cirrhosis of the liver, inhibitors of NF-κB-mediated gene expression can likewise represent an important therapeutic advance. There is evidence that NF-κB-controlled genes are induced as a result of oxidation reactions which lead to oxidative stress after reperfusion of ischemic tissue. In this way, an overexpression of cytokines and cell adhesion molecules in the ischemic tissue causes excessive recruitment of infiltrating alymphocytes. The recruited lymphocytes contribute causally to the tissue damage.

[0026] The involvement of NF-κB-controlled gene expression is evident in a number of neurodegenerative disorders. In particular in the case of nervous diseases in which the redox state of cells of the neuronal tissue is disturbed, a therapeutic benefit is ascribed to the selective inhibition of genes having an NF-κB binding sequence. A disturbed redox state of neuronal cells is assumed in the case of ainyotropic lateral sclerosis and in Down's syndrome.

[0027] It is therefore the general object of this invention to provides a method of inhibiting or preventing apoptosis in ischemic-reperfused myocardium using oligonucleotide decoys.

[0028] The present invention provides a method for inhibiting apoptosis in ischemic-reperfused myocardium by administering to a mammal an effective amount of oligonucleotide decoy, to reduce or prevent myocardial cell death in myocardial infarction. Furthermore, oligonucleotide decoys, either alone or conjugated to a polymeric vector or polyplex group, can be used to block apoptosis in situations of acute trauma, such as generalized trauma, global ischemia-reperfusion injury

occurring as a consequence of hemorrhagic shock, or spinal cord injury, thereby preventing cell death in organs such as the spinal cord.

[0029] We are using transcription factor decoys (including, but not limited to the one for NF-kB, disclosed), to block signaling and gene expression associated with pathogenesis. The data that we have pertains to blocking NF-kB in the heart, which we have shown is eficacious in reducing myocardial infarction. However, the concept that we wish to disclose takes this several steps further. First, we are and will use linear duplications or chains of these decoys, such that each strand contains a number of decoy transcription factor binding sites with more than 30, 35, 40 or more copies linked to stabilize the decoy and increases efficacy.

[0030] In another embodiment, we incorporate decoys for multiple transcription factors into one of these strands, such that the decoy can affect blockade of 2 or more transcription factors simultaneously in a cell. In another embodiment, we incorporate decoys for multiple transcription factors into one of these strands, such that the decoy can affect blockade of 3 or more transcription factors simultaneously in a cell.

[0031] Third, we use these strands, or the strands contained in a plasmid or other DNA vector (can include phage, viral or other DNA) to bind to the polyplexes to deliver the strands to the cytoplasm of the cell, to effect transcription factor blockade.

[0032] Preferably, the oligonucleotide decoy inhibits one or more transcription factor. More preferably, the oligonucleotide decoy inhibits NF-kB in addition to one or more transcription factor selected from the group consisting of AP-1, ATF2, ATF3, SP1 and related factors. By blocking key signaling molecules simultaneously has an additive or even synergistic therapeutic effect, particularly when the molecules chosen are key signaling hubs.

[0033] In another embodiment, the domains can include decoyts to one or more of NF-kB, iNOS, Cox2, metallothionein and heat shock protein 70.

[0034] The use of long chains of the binding sites to NF-kB and related genes, the decoy is able to bind much more NF-kB or related molecules per molecule and to effect binding to the polymers.

[0035] Generally, the term "oligonucleotide," refers to a short length of single-stranded polynucleotide chain. Oligonucleotides are typically less than 100 residues long (e.g., between 15 and 50), however, as used herein, the term is also intended to encompass longer polynucleotide chains. Oligonucleotides are often referred to by their length. For example a 22 residue oligonucleotide is referred to as a "22-mer". Oligonucleotides can form secondary and tertiary structures by self-hybridizing or by hybridizing to other polynucleotides. Such structures can include, but are not limited to, duplexes, hairpins, cruciforms, bends, and triplexes.

[0036] As used herein, the term "transcription factor" refers to proteins that interact with one another and RNA polymerase enzyme to modulate transcription. Transcription factors target genes by recognizing specific DNA regulatory sequences (e.g., enhancers) or other transcription factors. Transcription factors are often referred to as "trans-factors" that interact with "cis-elements" (e.g., enhancers) because they are typically produced from genes located distantly (trans) from their sites of regulation (cis). Some transcription factors are biologically active only when bound to another copy of itself (i.e., homodimers linked through "homodimerization domains") or to other transcription factors (i.e., heterodimers linked through "heterodimerization domains"). For most transcription factors, specific and distinct regions of the protein mediate DNA binding (i.e., "tDNA binding domains") and transcriptional activation (i.e., "activation domains").

[0037] As used herein, the terms "decoy" and "transcription factor decoy" refer to molecules that bind to or interact with transcription factors and prevent their binding to native enhancer sequences. Decoys include nucleic acid sequences, including, but not limited to, oligonucleotides that correspond to (i.e., are identical to or essentially identical to) the native enhancer. Such oligonucleotides include, but are not limited to, single stranded palindromic oligonucleotides comprising one or more repeats of the enhancer sequence, sense and antisense oligonucleotides comprising one or more repeats of the enhancer sequence, oligonucleotides that form hairpin structures such that a duplex binding site for the transcription factor is generated, and one or more oligonucleotides that form a cruciform structure such that one or more binding sites for the transcription factor are generated.

[0038] As used herein, the term "duplex," in reference to oligonucleotides, refers to regions that are double stranded through hybridization of complementary base pairs. The term "hairpin" refers to double-stranded nucleic acid structures formed by base-pairing between regions of the same strand of a nucleic acid molecule. The regions are arranged inversely and can be adjacent or separated by noncomplementary sequence (i.e., thus forming a loop structure or "stem-loop"). The term "cruciform" refers to structures formed in double-stranded nucleic acids by inverted repeats separated by a short sequence. Cruciform structures can be generated through the hybridization of two or more hairpin structures where the hairpin duplex and loop comprise the short sequence separating the inverted repeats. Cruciform structures can comprise one or more nucleic acid molecules.

[0039] In an alternate embodiment, the polynucleotide decoys of the invention comprise an internal oligonucleotide (I) having a length of X bases, where X is a number from about 10 to about 40, preferably 12 to 25, most preferably 14 to 20. The size of the I segments is bounded on the lower

end by their ability to maintain the relative binding affinity of the larger segments to, for example, transcription factors. The size of the I segments is bounded on the upper end by their ability to remain relatively insensitive to endonucleases. Thus, the length limits of the I segment of a decoy can be determined empirically by one of skill in the art.

[0040] In an alternate embodiment, the polynucleotide decoys of the invention further comprise cap or spacer oligonucleotides, having a length of from about 3 to about 24 bases, preferably 4 to 18 base most preferably 6 to 12 bases. Each of the cap or spacer oligonucleotides is comprised of bases that are unable to bind to any other base within the same cap oligonucleotide. Preferably each of the cap or spacer oligonucleotides consists of a single variety of nucleotide comprising a base selected from the group consisting of adenine, cytosine, thymidine, and modified nucleotides thereof.

[0041] In an alternate embodiment, the polynucleotide decoys of the invention comprise a formula comprising: (a) an internal oligonucleotide (I) having a length of X bases, where X is a number from about 14 to about 40; (b) a second complementary oligonucleotide (C_2) having a length of Z bases, where Z is a number greater than

[0042] Preferably, the domains (I) are covalently linked to the 5' end of the P1, the 3' end of the P1 is covalently linked to the 5' end of next I, the 3' end of the I is covalently linked to the 5' end of the P2, and the 3' end of the P2 is covalently linked to the 5' end of the next (I). In a specific embodiment, the polynucleotide comprises at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more domains (I) linked together with spacers. In one embodiment, the polynucleotide comprises at least two different domains (I) throughout the molecule.

[0043] The invention also provides for a purified decoy probe comprising a first nucleotide base recognition sequence region, wherein the first region binds

to a transcription factor; and an optionally present second nucleotide base recognition sequence region, provided that if the first region is nucleic acid and the second region is present, then the second region is either directly joined to the 5' end of the first region is joined to the 3' end or 5' end of the first region by a non-nucleotide linker, wherein the optionally present second region is present if the first region can be used to produce a functional double-stranded promoter sequence using a complementary oligonucleotide, further provided that if the first region is nucleic acid which can be used to produce the functional double-stranded promoter sequence using the complementary oligonucleotide, then the decoy probe does not have a nucleic acid sequence greater than about 10 nucleotides in length joined directly to the 3' end of the first region and the decoy probe does not have a terminal 3' OH group available to accept a nucleoside triphosphate in a polymerization reaction.

[0044] The diseases in which the therapeutic/prophylactic composition of the invention is indicated are NF-κB-associated diseases, that is to say diseases caused by the unwanted activation of genes under control of the transcriptional regulatory factor NF-κB, and among such diseases can be reckoned ischemic diseases, inflammatory diseases, autoimmune diseases, cancer metastasis and invasion, and cachexia. The ischemic disease includes ischemic diseases of organs (e.g. ischemic heart diseases such as myocardial infarction, acute heart failure, chronic heart failure, etc., ischemic brain diseases such as cerebral infarction, and ischemic lung diseases such as pulmonary infarction), aggravation of the prognosis of organ transplantation or organ surgery (e.g. aggravation of the prognosis of heart transplantation, cardiac surgery, kidney transplantation, renal surgery, liver transplantation, hepatic surgery, bone marrow transplantation, skin grafting, corneal transplantation, and lung transplantation), reperfusion disorders, and post-PTCA restenosis. The inflammatory disease mentioned above includes various inflammatory diseases such as nephritis, hepatitis, arthritis, etc., acute renal failure,

chronic renal failure, and arteriosclerosis, among other diseases. The autoimmune disease mentioned above includes but is not limited to rheumatism, multiple sclerosis, and Hashimoto's thyroiditis. Particularly the pharmaceutical composition containing the NF-κB decoy according to the present invention as an active ingredient is very suited for the therapy and prophylaxis of reperfusion disorders in ischemic diseases, aggravation of the prognosis of organ transplantation or organ surgery, post-PTCA restenosis, cancer metastasis and invasion, and cachexia such as weight loss following the onset of a cancer.

[0045] The NF-κB decoy that can be used in the present invention may be any compound that specifically antagonizes the NF-κB binding site of the chromosome and includes but is not limited to nucleic acids and their analogs. As preferred examples of the NF-κB decoy, the present invention may utilize NF-kB decoy comprising one or more copies of oligonucleotides CCTTGAAGGGATTTCCCTCC and GGAACTTCCCTAAAGGGAGG, preferably, the NF-kB decoy are described as oligonucleotides containing the nucleotide sequence of GGGATTTCCC. Preferably, the NF-kB decoy oligonucleotide is a double-stranded 22bp oligonucleotide (5'-AGTTGAGGGGACTTTCCCAGGC-3') (Promega)

[0046] The oligonucleotides may be DNAs or RNAs, and may contain modified nucleotides and/or pseudonucleotides. Furthermore, those oligonucleotides, variants thereof, or compounds containing any of them may be single-stranded or double-stranded and linear or cyclic. The variants are those involving mutations such as substitution, addition and/or deletion of any part of the above-mentioned sequence, and mean nucleic acids specifically antagonizing the binding site of chromosome to which NF-κB is conjugated. The more preferred NF-κB decoy includes double-stranded oligonucleotides each containing one or a plurality of the above nucleotide sequence and variants thereof. The oligonucleotide which can

be used in the present invention includes oligonucleotides modified so as to be less susceptible to biodegradation, such as those oligonucleotides containing the thiophosphoric diester bond available upon substitution of sulfur for the oxygen of the phosphoric diester moiety (S-oligo) and those oligonucleotides available upon substitution of a methyl phosphate group carrying no electric charge for the phosphoric diester moiety.

[0047] Regarding to a technology for producing the NF-κB decoy for use in the present invention, the conventional chemical or biochemical methods for synthesis can be utilized. When a nucleic acid, for instance, is to be used as the NF-κB decoy, the methods for nucleic acid synthesis which are commonly used in genetic engineering can be employed. For example, the objective decoy oligonucleotide can be directly synthesized on a DNA synthesizer. Or a nucleic acid or its fragments, each synthesized beforehand, can be amplified by PCR or using a cloning vector or the like. Furthermore, the desired nucleic acid can be obtained by such procedures as cleavage with restriction enzymes or the like and/or ligation by means of DNA ligase or the like. In order to obtain a decoy nucleotide which is more stable within cells, the base, sugar or/and phosphoric acid moieties of the nucleic acid may be alkylated, acylated, or otherwise chemically modified.

[0048] The pharmaceutical composition containing the NF-κB decoy as an active ingredient according to the present invention is not limited in form only if the active ingredient may be taken up by the cells in the affected site or the cells of the target tissue. Thus, the NF-κB decoy, either alone or in admixture with the common pharmaceutical carrier, can be administered orally, parenterally, topically or externally. The pharmaceutical composition may be provided in liquid dosage forms such as solutions, suspensions, syrups, liposomes, lotions, etc. or in solid dosage forms such as tablets, granules, powders, and capsules. Where necessary, those pharmaceutical compositions may be supplemented with various vehicles,

excipients, stabilizers, lubricants, and/or other conventional pharmaceutical additives, such as lactose, citric acid, tartaric acid, stearic acid, magnesium stearate, terra alba, sucrose, corn starch, talc, gelatin, agar, pectin, peanut oil, olive oil, caccao butter, ethylene glycol, and so on.

[0049] Particularly when a nucleic acid or a modification product thereof is used as the NF-κB decoy, the preferred dosage form includes those which are generally used in gene therapy, such as liposomes inclusive of membrane fusion liposomes utilizing Sendai virus and liposomes utilizing endocytosis, preparations containing cationic lipids such as Lipofectamine (Life Tech Oriental) or virosomes utilizing a retrovirus vector, adenovirus vector, or the like. Particularly preferred are membrane fusion liposomes.

[0050] The structure of such a liposomal preparation may be any of a large unilamellar vesicle (LUV), a multi-lamellar vesicle (MLV), and a small unilamellar vesicle (SUV). The approximate size of vesicles may range from 200 to 1000 nm for LUV, from 400 to 3500 nm for MLV, and from 20 to 50 nm for SUV but in the case of a membrane fusion liposomal preparation using Sendai virus, for instance, MLV with a vesicular system of 200-1000 nm in diameter is preferably employed.

[0051] There is no limitation on the technology for liposome production only if the decoy can be successfully entrapped in vesicles. Thus, such liposomes can be manufactured by the conventional techniques such as the reversed phase evaporation method (Szoka, F., et al: Biochim. Biophys. Acta, Vol. 601 559 (1980)), ether injection method (Deamer, D. W.: Ann. N. Y. Acad. Sci., Vol. 308 250 (1978)), and surfactant method (Brunner, J., et al: Biochim. Biophys. Acta, Vol. 455 322 (1976)), to name but a few examples.

[0052] The lipid that can be used for constructing a liposomal structure includes phospholipids, cholesterol and its derivatives, and nitrogen-containing lipids but phospholipids are generally preferred. The phospholipid that can

be used includes naturally-occurring phospholipids such as phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphatidic acid, cardiolipin, sphingomyelin, egg yolk lecithin, soybean lecithin, lysolecithin, etc., the corresponding phospholipids hydrogenated by the conventional method, and synthetic phospholipids such as dicetyl phosphate, distearoylphosphatidylcholine, dipalmitoylphosphatidylcholine, dipalmitoylphosphatidylethanolamine, dipalmitoylphosphatidylserine, eleostearoylphosphatidylcholine, eleostearoylphosphatidylethanolamine, eleostearoylphosphatidylserine, and so on.

[0053] The lipids inclusive of phospholipids can be used each alone or in a suitable combination. By using a lipid containing a positively-charged atomic group such as ethanolamine or choline within the molecule, the binding rate of an electrically negative decoy nucleotide can be enhanced. In addition to the principal phospholipid, various compounds such as cholesterol and its derivatives, stearylamine, -tocopherol, etc., which are known as liposome additives, can be added in the manufacture of liposomes.

[0054] To the resulting liposomes can be added a membrane fusion promoter such as Sendai virus, inactivated Sendai virus, a membrane fusion promoting protein purified from Sendai virus, polyethylene glycol, or the like can be added for assisting in the intracellular uptake by the cells at the affected site or of the target tissue.

[0055] There is no limitation on the decoy content of the pharmaceutical composition containing the decoy as an active ingredient only if the decoy is contained in amounts effective to control NF-κB-associated diseases. Thus, the decoy content can be liberally selected according to the disease to be controlled, the target site, dosage form, and dosage schedule.

[0056] The pharmaceutical composition containing the decoy as an active ingredient as provided in the above manner can be administered by various methods according to the type of disease and the kind of decoy contained. Taking ischemic diseases, inflammatory diseases, autoimmune diseases, cancer metastasis or invasion, and cachexia as examples, the composition can be infused intravascularly, applied directly to the affected area, injected into the lesion, or administered into the regional blood vessel in the affected region. As a further specific example, when PTCA is performed for infarction of an organ, the pharmaceutical composition can be administered into the local blood vessel concurrently with the operation or pre- and postoperatively. For organ transplantation, the graft material can be previously treated with the composition of the invention. Furthermore, in the treatment of osteoarthritis or rheumatism, the composition can be directly injected into the joint.

[0057] The dosage of the decoy is selected with reference to the patient's age and other factors, type of disease, the kind of decoy used, etc. but for intravascular, intramuscular, or intraarticular administration, for instance, a unit dose of 10-10,000 nmoles can generally be administered once to a few times daily.

[0058] As used herein, the term "procedural vascular trauma" includes the effects of surgical/mechanical interventions into mammalian vasculature, but does not include vascular trauma due to the organic vascular pathologies listed hereinabove.

[0059] Thus, procedural vascular traumas within the scope of the present treatment method include (1) organ transplantation, such as heart, kidney, liver and the like, e.g., involving vessel anastomosis; (2) vascular surgery, such as coronary bypass surgery, biopsy, heart valve replacement, atheroectomy, thrombectomy, and the like; (3) transcatheter vascular therapies (TVT) including angioplasty, e.g., laser angioplasty and PTCA procedures discussed hereinbelow, employing balloon catheters, and

indwelling catheters; (4) vascular grafting using natural or synthetic materials, such as in saphenous vein coronary bypass grafts, dacron and venous grafts used for peripheral arterial reconstruction, etc.; (5) placement of a mechanical shunt, such as a PIFE hemodialysis shunt used for arteriovenous communications; and (6) placement of an intravascular stent, which may be metallic, plastic or a biodegradable polymer. See U.S. patent application Ser. No. 08/389,712, filed Feb. 15, 1995, which is incorporated by reference herein. For a general discussion of implantable devices and biomaterials from which they can be formed, see H. Kambic et al., "Biomatedals in Artificial Organs", Chem. Eng. News. 30 (Apr. 14, 1986), the disclosure of which is incorporated by reference herein.

[0060] The present invention generally relates to coronary heart attacks and cardiac surgery. More particularly, the invention is related to the use of oligonucleotide decoy as a protective agent during cardiac surgery and during the ischemia/reperfusion phases of acute myocardial infarction (coronary heart attack).

[0061] The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the Examples included herein. The NFkB associated polynucleotides and polypeptides are sometimes refered to herein as "NFkB modulatory" polynucleotides and polypeptides. Likewise, all references to "NFkB associated polynucleotides and polypeptides" shall be construed to apply to "NFkB modulatory polynucleotides and polypeptides".

[0062] The invention provides the polynucleotide and polypeptide sequences of genes that are believed to be associated with the NF-kB pathway. As referenced herein, members of the NFkB family are transcription factors that are critical regulators of inflammatory and stress responses. Thus, the polynucleotide and polypeptides of the present invention may also be represent critical regulators of inflammatory and stress responses.

[0063] In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

[0064] In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

[0065] Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373, preferably a Model 3700, from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by

translation of a DNA sequence determined above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

[0066] Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C. in a solution comprising 6.times.SSPE (20.times.SSPE=3M NaCl; 0.2M NaH2PO4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C. with 1.times.SSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5.times.SSC).

[0067] Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence

listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

[0068] The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

[0069] The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the

amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

[0070] The term "organism" as referred to herein is meant to encompass any organism referenced herein, though preferably to eukaryotic organisms, more preferably to mammals, and most preferably to humans.

[0071] As used herein the terms "modulate" or "modulates" refer to an increase or decrease in the amount, quality or effect of a particular activity, DNA, RNA, or protein. The definition of "modulate" or "modulates" as used herein is meant to encompass agonists and/or antagonists of a particular activity, DNA, RNA, or protein.

[0072] Specifically, the invention provides methods for using the polynucleotides and polypeptides of the invention to identify orthologs, homologs, paralogs, variants, and/or allelic variants of the invention. Also provided

are methods of using the polynucleotides and polypeptides of the invention to identify the entire coding region of the invention, non-coding regions of the invention, regulatory sequences of the invention, and secreted, mature, pro-, prepro-, forms of the invention (as applicable).

[0073] In preferred embodiments, the invention provides methods for identifying the glycosylation sites inherent in the polynucleotides and polypeptides of the invention, and the subsequent alteration, deletion, and/or addition of the sites for a number of desirable characteristics which include, but are not limited to, augmentation of protein folding, inhibition of protein aggregation, regulation of intracellular trafficking to organelles, increasing resistance to proteolysis, modulation of protein antigenicity, and mediation of intercellular adhesion.

[0074] In further preferred embodiments, methods are provided for evolving the polynucleotides and polypeptides of the present invention using molecular evolution techniques in an effort to create and identify novel variants with desired structural, functional, and/or physical characteristics.

[0075] As used herein the terms "modulate" or "modulates" refer to an increase or decrease in the amount, quality or effect of a particular activity, DNA, RNA, or protein.

[0076] While the NFkB-associated sequences are likely to comprise representatives from a number of protein families and classes (such as GPCRs, transcription factors, ion channels, proteases, nucleases, secreted proteins, nuclear hormone receptors, etc.), their biological activity will likely not be exactly the same as NFkB (e.g., a transciption factor). Rather the NFkB associated polynucleotides and polypeptides of the present invention are believed to represent either direct, or indirect, participating members of the NFkB pathway. Therefore, it is expected that the NFkB associated polynucleotides and polypeptides of the present invention, including agonists, antagonists, or fragments thereof, will be capable of

providing at least some of the therapeutic benefits afforded by modulators of NFkB, and potentially NFkB itself, upon administration to a patient in need of treatment. The present invention also encompasses antagonists or agonists of the polynucleotides and polypeptides, including fragments thereof, and their potential utility in modulating NFkB modulators, and potentially NFkB itself.

[0077] Modulating the activity of the NFkB associated genes of the present invention may result in fewer toxicities than the drugs, therapies, or regimens presently known to regulate NF-kappaB itself. Such NF-kappaB inhibitors include the following, non-limiting examples: NFkB decoy oligonucleotide-HVJ liposomes complex (Dainippon); gene therapy-based implantation of the I kappa B gene into donor organs ex vivo (Novartis; EP699977); drugs designed to block IkappaBalpha-directed ubiquitination enzymes resulting in more specific suppression of NF-kB activation (Aventis); declopramide (OXIGENE; CAS Registry Number: 891-60-1); IPL-550260 (Inflazyme); IPL-512602 (Inflazyme); KP-392 (Kinetek); R-flurbiprofen (Encore Pharmaceuticals; E-7869, MPC-7869; (1,1'-Biphenyl)-4-acetic acid, 2-fluoro-alpha-methyl; CAS Registry Number: 5104-49-4); drugs disclosed in U.S. Pat. Nos. 5,561,161 and 5,340,565 (OXiGENE); dexlipotam (Asta Medica); RIP-3 Rigel (Rigel; Pharmaprojects No. 6135); tyloxapol Discovery (Discovery Laboratories; SuperVent; 4-(1,1,3,3-Tetramethylbutyl)phenol polymer with formaldehyde andoxirane; CAS Registry Number: 25301-02-4); IZP-97001 (Inflazyme); IZP-96005 (Inflazyme); IZP-96002 (Inflazyme); sortac (Inflazyme; IPL-400); BXT-51072 (OXIS; 2H-1,2-Benzoselenazine, 3,4-dihydro-4,4-dimethyl-; CAS Registry Number: 173026-17-0); SP-100030 (Celgene; 2-chloro-N-(3,5-di(trifluoromethyl)phenyl)-4-(trifluo- romethyl)pyrimidine-5-carboxamide); IPL-576092 (Inflazyme; Stigmastan-15-one, 22,29-epoxy-3,4,6,7,29-pentahydroxy-, (3alpha,4beta,5alpha, 6alpha,7beta, 14beta,22S); CAS Registry Number: 137571-30-3; U.S. Pat. No.

6,046,185); P54 (Phytopharm); Interleukin-10 (Schering-Plough;SCH 52000; Tenovil; rI-10; rhIL-10; CAS Registry Number: 149824-15-7); and antisense oligonucleotides PLGA/PEG microparicles.

[0078] The NFkB associated polynucleotides and polypeptides of the present invention, including agonists, and/or fragments thereof, have uses that include detecting, prognosing, treating, preventing, and/or ameliorating the following diseases and/or disorders: immune disorders, inflammatory disorders, aberrant apoptosis, hepatic disorders, Hodgkins lymphomas, hematopoietic tumors, hyper-IgM syndromes, hypohydrotic ectodermal dysplasia, X-linked anhidrotic ectodermal dysplasia, Immunodeficiency, al incontinentia pigmenti, viral infections, HIV-1, HTLV-1, hepatitis B, hepatitis C, EBV, influenza, viral replication, host cell survival, and evasion of immune responses, rheumatoid arthritis inflammatory bowel disease, colitis, asthma, atherosclerosis, cachexia, euthyroid sick syndrome, stroke, and EAE.

[0079] Alternatively, antagonists and/or fragments of the NFkB associated polynucleotides and polypeptides of the present invention have uses that include detecting, prognosing, treating, preventing, and/or ameliorating the following diseases and/or disorders: immune disorders, inflammatory disorders, aberrant apoptosis, hepatic disorders, Hodgkins lymphomas, hematopoietic tumors, hyper-IgM syndromes, hypohydrotic ectodermal dysplasia, X-linked anhidrotic ectodermal dysplasia, immunodeficiency, al incontinentia pigmenti, viral infections, HIV-1, HTLV-1, hepatitis B, hepatitis C, EBV, influenza, viral replication, host cell survival, and evasion of immune responses, rheumatoid arthritis, inflammatory bowel disease, colitis, asthma, atherosclerosis, cachexia, euthyroid sick syndrome, stroke, and EAE.

We claim:

1. A method for treatment of NF-κB-associated diseases which comprises administering to an animal an effective amount of a polynucleotide NF-κB chromosomal binding site decoy which antagonizes NF-κB-mediated transcription of a gene located downstream of a NF-κB binding site wherein the polynucleotide comprises one or more copy of the oligonucleotide decoy.
2. The method according to claim 1 wherein the NF-κB-associated disease is selected from the group consisting of; an ischemic disease, an inflammatory disease, and an autoimmune disease.
3. The method according to claim 1 wherein the NF-κB-associated disease is an ischemic disease.
4. The method according to claim 1 wherein the NF-κB-associated disease is selected from the group consisting of; a reperfusion disorder in ischemic disease, aggravation of a prognosis of an organ transplantation, aggravation of a prognosis of an organ surgery, a post-PTCA restinosis.
5. The method according to claim 1 wherein the NF-κB-associated disease is selected from the group consisting of; a reperfusion disorder in ischemic heart disease, aggravation of a prognosis of a heart transplantation, aggravation of a prognosis of a heart surgery, and post PTCA restinosis.

6. The method according to claim 1 wherein the NF-κB-associated disease is selected from the group consisting of; a cancer metastasis a cancer invasion, and cachexia.

7. A method of treating a nuclear factor κB-dependent disease selected from the group consisting of immunological disorders, septic shock, transplant rejection, radiation damage, reperfusion injuries after ischemia, arteriosclerosis and neurodegenerative diseases, comprising administering to a mammal in need of such treatment an effective amount of an oligonucleotide decoy.

8. The method of claim 7 wherein the oligonucleotide decoy is delivered by a polymeric vector.

9. The method of claim 7 wherein the nuclear factor-κB-dependent disease is an immunological disorder.

10. The method of claim 7 wherein the nuclear factor-κB-dependent disease is septic shock.

11. The method of claim 7 wherein the nuclear factor-κB-dependent disease is transplant rejection.

12. The method of claim 7 wherein the nuclear factor-κB-dependent disease is radiation damage.

13. The method of claim 7 wherein the nuclear factor-κB-dependent disease is reperfusion injury after ischemia.

14. The method of claim 7 wherein the nuclear factor-κB-dependent disease arteriosclerosis.

15. The method of claim 1 wherein the nuclear factor-κB-dependent disease is a neurodegenerative disease.

16. The method according to claim 1 wherein the administering inhibits apoptosis in ischemic-reperfused myocardium.

17. The method according to claim 1 wherein the administering inhibits apoptosis in ischemic-reperfused brain, reducing neuronal cell death in stroke.

18. The method according to claim 1 wherein the administering inhibits apoptosis in the failing heart, reducing apoptosis cell death in congestive heart failure and cardiomyopathy.

19. A therapeutic method comprising treating non-aortal procedural vascular trauma comprising administering to a mammal, subjected to the procedural vascular trauma, an effective protective amount of an oligonucleotide decoy, or a pharmaceutically acceptable salt thereof.

20. The method of claim 1 wherein the oligonucleotide decoy is complexed with a polymeric vector.

21. The method of claim 1 wherein the administration is before or after the procedure, or both before and after the procedure.

22. The method of claim 1 wherein the administration is in a series of spaced doses.

23. The method of claim 1 wherein the administration is parenteral.

24. The method of claim 1 wherein the administration is oral.

25. The method of claim 1 wherein the administration is systemic.

26. The method of claim 1 wherein the administration is localized at the site of the vascular trauma.

27. The method of claim 1 wherein the procedure is organ transplantation.

28. The method of claim 10 wherein the organ is kidney.

29. The method of claim 10 wherein the organ is a liver.

30. The method of claim 1 wherein the procedure is a biopsy.

31. The method of claim 1 wherein the procedure is thrombectomy.

32. The method of claim 1 wherein the procedure is a vascular bypass graft.

33. The method of claim 1 wherein the cells comprise ocular tissue.

1) A method of gene delivery wherein the targeted gene is delivered via a polyamide obtained by the copolymerization of an alkylpolyamine and a suitable co-monomer, selected from the group consisting of monosaccarides, disaccharides and polysaccarides.

2) A gene delivery vehicle used for treatment of heart disease, wherein the delivery vehicle comprises a polyamide obtained by the copolymerization of an alkylpolyamine and a suitable aldose.

3) A nontoxic polymeric delivery vector obtained by the copolymerization of an alkylpolyamine and a suitable co-monomer, selected from the group consisting of monosaccharides, disaccharides and polysaccharides.

4) A nontoxic polymeric delivery vector that delivers dsDNA decoys to inactivate NF-κB in myocardial cells, wherein the delivery vector is a polyamide formed by reacting a diamine co-monomer with a suitable carbohydrate.

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/042948

International filing date: 20 December 2004 (20.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/574,131
Filing date: 25 May 2004 (25.05.2004)

Date of receipt at the International Bureau: 04 July 2005 (04.07.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse